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IL-1β mediates chronic intestinal inflammation by promoting the accumulation of IL-17A secreting innate lymphoid cells and CD4+ Th17 cells

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Although very high levels of interleukin (IL)-1β are present in the intestines of patients suffering from inflammatory bowel diseases (IBD), little is known about the contribution of IL-1β to intestinal pathology. Here, we used two complementary models of chronic intestinal inflammation to address the role of IL-1β in driving innate and adaptive pathology in the intestine. We show that IL-1β promotes innate immune pathology in *Helicobacter hepaticus*–triggered intestinal inflammation by augmenting the recruitment of granulocytes and the accumulation and activation of innate lymphoid cells (ILCs). Using a T cell transfer colitis model, we demonstrate a key role for T cell–specific IL-1 receptor (IL-1R) signals in the accumulation and survival of pathogenic CD4+ T cells in the colon. Furthermore, we show that IL-1β promotes Th17 responses from CD4+ T cells and ILCs in the intestine, and we describe synergistic interactions between IL-1β and IL-23 signals that sustain innate and adaptive inflammatory responses in the gut. These data identify multiple mechanisms through which IL-1β promotes intestinal pathology and suggest that targeting IL-1β may represent a useful therapeutic approach in IBD.

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Abbreviations used: 7AAD, 7-aminoactinomycin; CD, Crohn's disease; cLPL, colonic lamina propria leukocyte; DSS, dextran sulfate sodium; EAE, experimental autoimmune encephalomyelitis; IBD, inflammatory bowel disease; ILC, innate lymphoid cell; MLN, mesenteric lymph node; n/a, nothing added; ND, not detectable; UC, ulcerative colitis.

Inflammatory bowel disease (IBD), clinically comprising Crohn's disease (CD), and ulcerative colitis (UC) are chronic inflammatory disorders of the gut with complex etiologies (Kaser et al., 2010). The understanding of pathogenic mechanisms underlying the development of IBD has aided the development of novel biological therapies. Agents that block the TNF pathways have been particularly successful in IBD therapy (Melmed and Targan, 2010). However, a large proportion of patients either fail to respond or develop tolerance to TNF therapy, highlighting the need for new therapeutic targets (Baumgart and Sandborn, 2007).

IL-1β is a proinflammatory cytokine with a wide range of systemic and local effects. Primarily produced by innate leukocytes, IL-1β can modulate the function of both immune and nonimmune cells. Stimulation with IL-1β promotes the activation and effector functions of dendritic cells, macrophages, and neutrophils (Dinarello, 1996). Moreover, IL-1β can induce neutrophilia and promote neutrophil migration (Dinarello, 2009). IL-1β promotes T cell activation and survival (Ben-Sasson et al., 2009) and has recently been shown to act in concert with other proinflammatory cytokines to promote the differentiation of CD4+ Th17 cells (Sutton et al., 2006, 2009; Acosta-Rodriguez et al., 2007; Chung et al., 2009). The potent inflammatory activity of IL-1β is reflected by the tight mechanisms in place to regulate its secretion. IL-1β is translated as an inactive 31 kD precursor (pro-IL-1β) after TLR stimulation, which is cleaved into its activated 17 kD form by caspase-1, also known as interleukin-1β converting enzyme (ICE; Thornberry et al., 1992). Activation of caspase-1 relies on the formation of a multimolecular scaffold known as the inflammasome,

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which is triggered by the activation of intracellular NOD-like receptors (NLR) by endogenous or exogenous danger signals (Martinon et al., 2009). Upon secretion into the extracellular space, IL-1β binds and signals via the IL-1 receptor 1 (IL-1R1), which is expressed on a wide range of cell types, including epithelial and endothelial cells, hepatocytes, and innate and adaptive leukocytes (Sims and Smith, 2010). Regulatory mechanisms downstream of IL-1β secretion are also in place to limit the action of IL-1β, including the production of a natural antagonist for its receptor, IL-1RA, and the expression of a decoy receptor, IL-1R2 (Dinarello, 1996).

Several clinical studies have reported high levels of IL-1β secretion by colon lamina propria monocytes from patients with active IBD (Satsangi et al., 1987; Mahida et al., 1989; Ligumsky et al., 1990; Reinecker et al., 1993; McAlindon et al., 1998). IL-1β levels in the colon correlated with disease activity and high levels of IL-1β were associated with active lesions (Casini-Raggi et al., 1995; Ludwiczek et al., 2004), suggesting an important role of this cytokine in promoting localized inflammation. High levels of colonic IL-1β are also a feature of many animal models of colitis (Cominelli et al., 1990; Okayasu et al., 1990; McCall et al., 1994), and treatment with IL-1–blocking agents has been successful in ameliorating acute models of intestinal injury and inflammation (Thomas et al., 1991; Cominelli et al., 1992; Siegmund et al., 2001). Furthermore, different genetic lesions associated with IBD development in animal models are associated with increased IL-1β. For example, macrophages from knock-in mice bearing the most common CD-associated variant of the *Nod2* gene produced high levels of IL-1β after stimulation with muramyl dipeptide (MDP; Maeda et al., 2005). These NOD2 mutant mice also developed exacerbated disease in response to acute dextran sulfate sodium (DSS)–induced intestinal injury, which was significantly ameliorated by the administration of recombinant IL-1RA (Maeda et al., 2005). In addition, conditional deletion of the CD-linked autophagy gene *Atg16l1* in the hematopoietic system of mice resulted in increased IL-1β production after LPS stimulation and increased susceptibility to DSS-mediated intestinal injury, a phenotype reversed by co-treatment with αIL-18 and αIL-1β antibodies (Saitoh et al., 2008). The importance of IL-1β in modulating intestinal inflammation has been confirmed by infection studies, as blocking IL-1β ameliorated inflammatory pathology in both *Clostridium difficile*–associated colitis and *Salmonella typhimurium*–induced enteritis (Müller et al., 2009; Ng et al., 2010).

Although IL-1β has been linked to Th17 cell responses, the role of IL-17A in the development of intestinal inflammation is controversial. In acute models of intestinal injury, IL-17A plays a disease protective role, with *Il17a*$^{-/-}$ mice showing increased pathology and leukocyte infiltration after DSS administration (Ogawa et al., 2004). However, studies in chronic inflammatory models have highlighted a more complex role for IL-17A. Studies from our laboratory demonstrated a pathogenic role for IL-17A in *Helicobacter hepaticus* (*H. hepaticus*)–driven innate immune IBD (Buonocore et al., 2010). Similarly, administration of an αIL-17A antibody ameliorated the spontaneous colitis developed by mice bearing a conditional deletion of the transcription factor Stat3 in regulatory Foxp3^{+} T cells (Chaudhry et al., 2009). Th17 cells are enriched in the inflamed gut both in animal models and in humans (Fujino et al., 2003; Nielsen et al., 2003; Hue et al., 2006; Kullberg et al., 2006) and T cells lacking the transcription factor RORγ, that directs Th17 differentiation, could not transfer colitis to C57BL/6 *Rag1*$^{-/-}$ mice (Leppkes et al., 2009). However, T cell–derived IL-17A is not absolutely required for the development of intestinal pathology in T cell transfer models of colitis and it has been proposed that T cell–derived IL-17A and IL-17F might play a redundant role in driving intestinal inflammation (Izcue et al., 2008; Leppkes et al., 2009; O'Connor et al., 2009). These conflicting results might be explained by an as yet undiscovered additional pathogenic function of Th17 cells. Alternatively, a complex network of proinflammatory cells may contribute to IL-17A–mediated pathology in vivo (Littman and Rudensky, 2010).

In this study, we aimed to assess the role of IL-1β in chronic intestinal inflammation. As a result of the pluripotent activity of IL-1β, we used complementary animal models of chronic colitis to selectively analyze the effects of IL-1β on adaptive and innate immune-mediated intestinal inflammation. Our results show that IL-1β signals are required for the development of severe inflammation in both T cell–independent and T cell–mediated colitis. Moreover, we identified key mechanisms underlying the pathogenic function of IL-1β, including a central role for this cytokine in promoting the accumulation of IL-17A–producing innate and adaptive immune cells.

RESULTS

IL-1β plays a key role in innate intestinal inflammation

To specifically analyze the role of IL-1β in modulating innate inflammatory responses in the intestine, we infected T cell– and

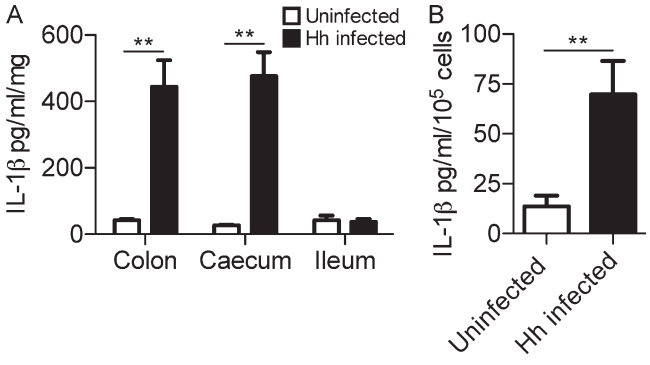

Figure 1. *H. hepaticus*–induced innate immune driven typhlocolitis is associated with IL-1β secretion. 129SvEv *Rag2*$^{-/-}$ mice were infected with *H. hepaticus* and sacrificed >8 wk after infection. (A) IL-1β secretion from organ explants from colon, cecum, and ileum incubated overnight in complete medium. Results are shown as mean ± SEM (n = 3 for uninfected control and n = 14 for *H. hepaticus*–infected mice, pooled from 2 independent experiments). (B) IL-1β levels in the supernatants of cLPLs cultured overnight in complete media. Results are shown as mean ± SEM (n = 7 for uninfected controls and n = 22 for *H. hepaticus*–infected mice, pooled from 3 separate experiments). **, $P < 0.01$.

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B cell–deficient 129SvEv *Rag2*$^{-/-}$ mice with *H. hepaticus*, which results in the development of colonic and cecal inflammation that is entirely dependent on innate immunity (Erdman et al., 2003; Maloy et al., 2003). We first examined whether *H. hepaticus*–induced intestinal inflammation was associated with increased levels of active IL-1β by analyzing the levels of IL-1β secretion by organ explants from the gastrointestinal tract of *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice. Intestinal inflammation in the colon and cecum of *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice was associated with high levels of secreted IL-1β (Fig. 1 A). In contrast, no increase in IL-1β levels was observed in the ileum of *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice (Fig. 1 A). Given that both colonization and *H. hepaticus*–induced inflammation occur primarily in the colon and cecum, this result suggests that IL-1β secretion is directly linked to local intestinal inflammation. Innate leukocytes appear to be the major source of IL-1β secretion in the lamina propria in IBD patients (Mahida et al., 1989; McAlindon et al., 1998). We observed high levels of IL-1β secretion by purified colonic lamina propria leukocytes (cLPLs) of *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice (Fig. 1 B), confirming that chronic intestinal inflammation correlates with increased local secretion of IL-1β by innate leukocytes.

To formally assess the requirement for IL-1β in *H. hepaticus*–induced innate intestinal inflammation, we blocked the activity of IL-1β with a neutralizing monoclonal antibody (αIL-1β). Treatment of *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice with αIL-1β resulted in significant attenuation of colitis (Fig. 2, A–C), without affecting *H. hepaticus* colonization (unpublished data). Although cecal inflammation was not significantly attenuated (not depicted), hepatic inflammation was also reduced by administration of αIL-1β, as illustrated by the decreased number of inflammatory foci (Fig. 2 C).

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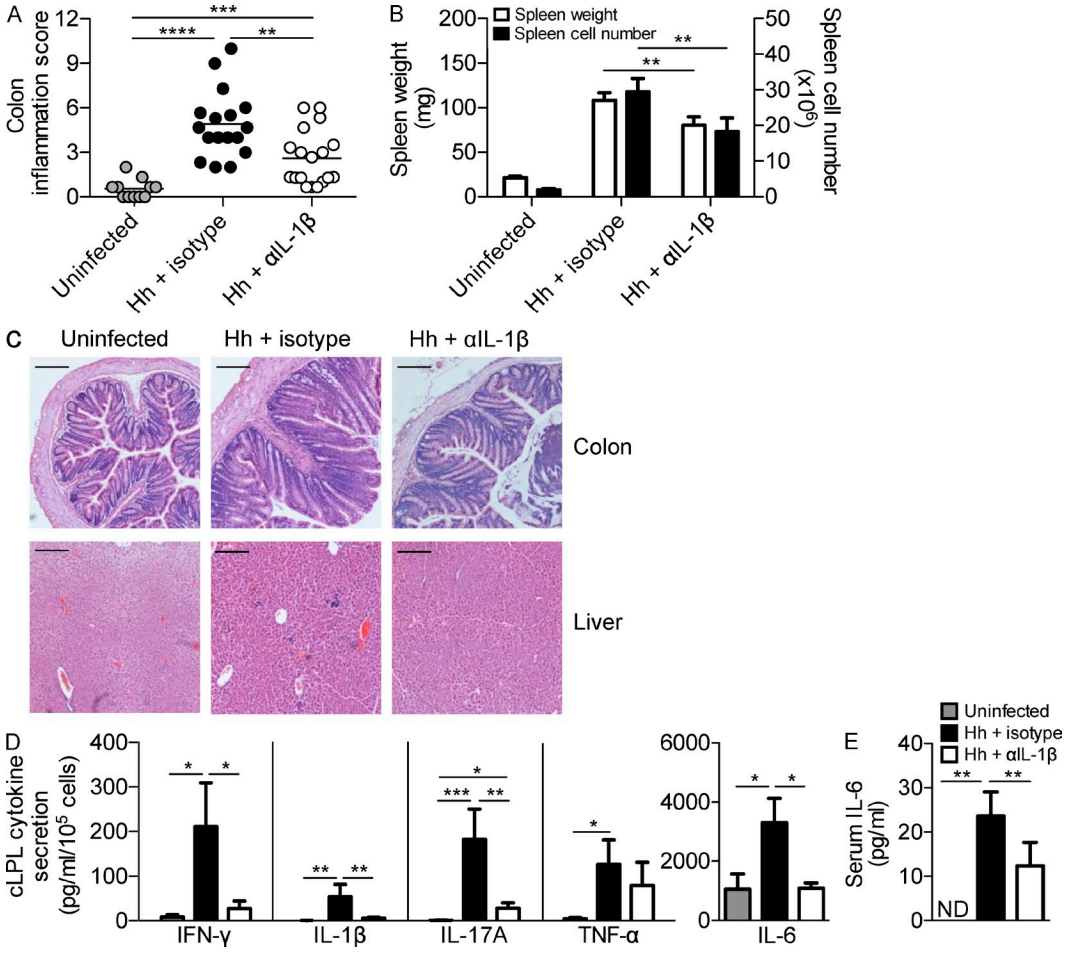

Figure 2. IL-1β drives innate immune colitis. 129SvEv *Rag2*$^{-/-}$ mice were infected with *H. hepaticus*, treated weekly with 1 mg of αIL-1β antibody or isotype control (i.p.), and sacrificed after 8 wk of infection. (A) Scores of colon inflammation. Each symbol represents an individual mouse, and data are pooled from four independent experiments. Bars represent the mean histological score for each group. (B) Spleen weights and splenocyte numbers. (C) Representative photomicrographs of mid-colon and liver sections. Bars, 200 µm. (D) Colonic lamina propria leukocytes were isolated from the described mice groups and cytokine secretion after overnight culture in complete media was determined. Data are shown as mean ± SEM from 3 pooled independent experiments (n = 9–16). (E) Cytokine concentration in blood serum (mean ± SEM, n = 6–10 from 2 pooled independent experiments). ND, not detectable; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

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Moreover, systemic inflammation was also reduced after IL-1β blockade, as shown by decreased splenomegaly and spleen cellularity in αIL-1β–treated animals (Fig. 2 B). These results identify a role for IL-1β in promoting intestinal and systemic innate inflammation after *H. hepaticus* infection. To further characterize the effect of blocking IL-1β, we examined the levels of proinflammatory cytokines secreted by purified cLPLs from the different treatment groups (Fig. 2 D). As expected (Hue et al., 2006), we observed an increase in proinflammatory cytokine production by cLPLs from *H. hepaticus*–infected colitic mice. After administration of αIL-1β, we observed significantly decreased levels of IL-6, IFN-γ, IL-17A, and IL-1β when compared with isotype control–treated animals (Fig. 2 D). In addition, significantly increased levels of IL-6 were detected in the serum of *H. hepaticus*–infected animals, and these were markedly reduced by αIL-1β treatment (Fig. 2 E). These results support a crucial role of IL-1β in promoting proinflammatory cytokine cascades in innate immune colitis.

IL-1β blockade decreases granulocyte recruitment and accumulation of IL-17A–producing innate lymphoid cells in the colon

H. hepaticus–induced innate colitis is associated with pronounced accumulation of proinflammatory $CD11b^{+}Gr1^{Hi}$ granulocytes in the colon and spleen of infected mice (Hue et al., 2006). Thus, we isolated leukocytes from different tissues of *H. hepaticus*–infected αIL-1β–treated and isotype control-treated 129SvEv *Rag2*$^{-/-}$ mice and analyzed the frequency of granulocytes by flow cytometry. As expected, *H. hepaticus*–mediated inflammation was associated with an increase in the frequency of $CD11b^{+}Gr1^{Hi}$ cells in the colon and spleen (Fig. 3, A and B). Blocking IL-1β in *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ resulted in a decrease in the frequency of $CD11b^{+}Gr1^{Hi}$ granulocytes in the colon, although it did not affect frequencies in the spleen (Fig. 3, A and B). IL-1β promotes neutrophil recruitment by inducing the expression of C-X-C chemokines in vivo (Calkins et al., 2002). Accordingly, we observed a significant down-regulation of *Cxcl1* (KC), *Cxcl2* (MIP2α), and *Cxcl5* (ENA78) in the colon of *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice treated with αIL-1β, compared with *H. hepaticus*–infected mice receiving an isotype control (Fig. 3 C). These data highlight an intestine-specific role of IL-1β in promoting the recruitment of proinflammatory innate immune cell populations through the induction of chemokine expression.

We recently identified a novel population of $Ror\gamma t^{+}$ $Sca1^{+}$ $Thy1.2^{Hi}$ innate lymphoid cells (ILCs) that play an important role in innate immune-mediated intestinal inflammation (Buonocore et al., 2010). Expression analysis revealed that ILCs isolated from the large intestine of *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice express high levels of the IL-1 receptor IL-1R1 (Fig. 4 A), suggesting that ILCs can directly be modulated by IL-1β in vivo. Thus, we analyzed the number and function of these cells in the colon after αIL-1β treatment compared with isotype control–treated mice. We observed significantly decreased numbers of ILCs in the colons of *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice that received αIL-1β compared with those treated with an isotype control (Fig. 4 B). During *H. hepaticus*–induced intestinal inflammation, ILCs produce the proinflammatory cytokines IL-17A and IFN-γ, and these responses are enhanced

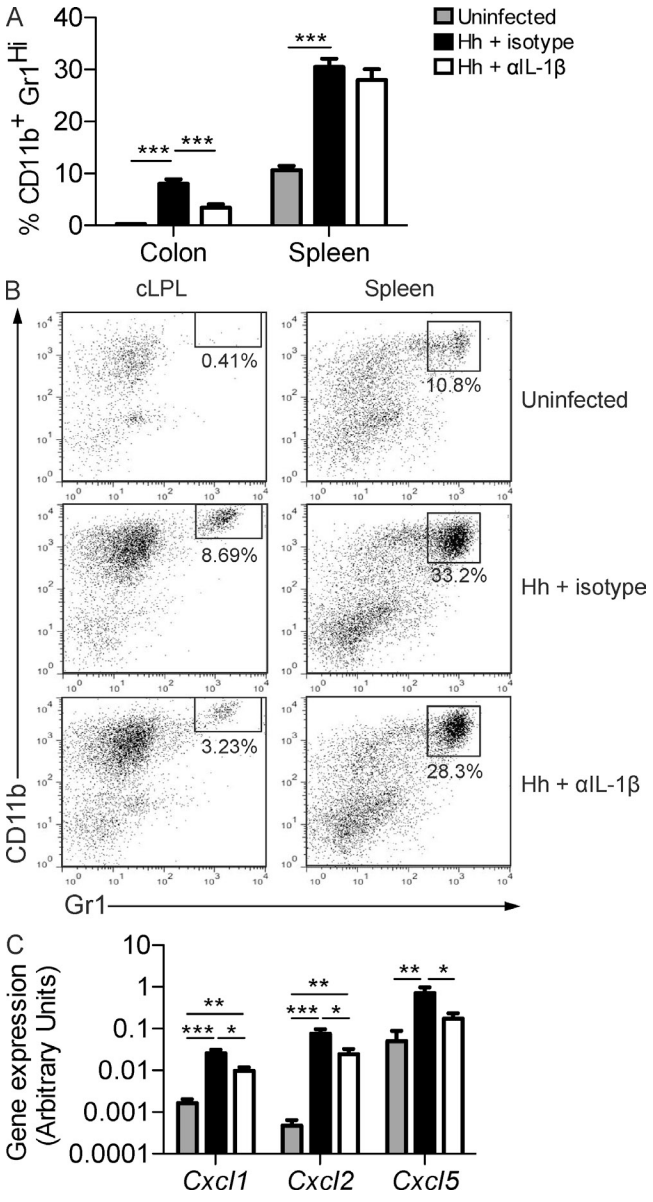

Figure 3. Blocking IL-1β impairs the accumulation of proinflammatory $CD11b^{+}Gr1^{Hi}$ granulocytes in the inflamed colon. 129SvEv *Rag2*$^{-/-}$ mice were infected with *H. hepaticus*, treated weekly with 1 mg of αIL-1β antibody or isotype control (i.p.), and sacrificed after 8 wk of infection. (A) Leukocytes were isolated from the colon and spleen, and the frequency of $CD45^{+}CD11b^{+}Gr1^{Hi}$ cells was assessed by flow cytometry (mean ± SEM from 3 pooled independent experiments, $n = 9–16$). (B) Representative FACS plots from the data presented in A (gated on $CD45^{+}$ cells). (C) Colon chemokine expression as evaluated by qRT-PCR on total colon homogenates (mean ± SEM, $n = 6–12$ from 2 pooled independent experiments). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

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after stimulation with IL-23 (Buonocore et al., 2010). Intracellular cytokine staining revealed that the observed decrease in ILC number in the colon after αIL-1β treatment was accompanied by a significant decrease in IL-17A–producing ILCs and by a reduction in the number of IFN-γ–producing ILCs (Fig. 4 C). These results indicate a role of IL-1β in promoting the accumulation and activation of ILCs in the colon. As we have shown that ILCs are the major IL-23–responsive population in this innate colitis model (Buonocore et al., 2010), we cultured cLPLs from αIL-1β–treated, *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice in the presence of IL-23. Blockade of IL-1β in vivo resulted in decreased production of both IL-17A and IFN-γ by IL-23–stimulated cLPLs in vitro (Fig. 4 D). Consistent with this reduced response to IL-23, cLPLs isolated from αIL-1β–treated *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ exhibited reduced *IL23r* expression compared with those isolated from mice receiving an isotype control (Fig. 4 E). To test whether IL-1β directly regulated *IL23r* expression by ILCs, FACS sorted ILCs from the large intestines of *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice were cultured with IL-1β and *IL23r* expression assayed using qRT-PCR. ILCs that were cultured with IL-1β expressed higher levels of *IL23r* than those cultured in medium alone, whereas the levels of expression of the transcription factor Rorγt did not change upon culture with IL-1β (Fig. 4 F). Conversely, culture with IL-23 did not significantly enhance *Il1r1* expression by ILCs (Fig. 4 G). Collectively, these data indicate that IL-1β plays multiple roles in promoting innate immune responses in the colon by modulating recruitment, accumulation, and function of key proinflammatory cell types.

T cell–specific IL-1R1 signaling drives T cell–mediated intestinal inflammation

We next addressed whether IL-1β could also promote deleterious adaptive immune responses within the intestine. To this end, we used the T cell transfer model of colitis in which transfer of naive CD4$^+$ T cells to a lymphopenic host results

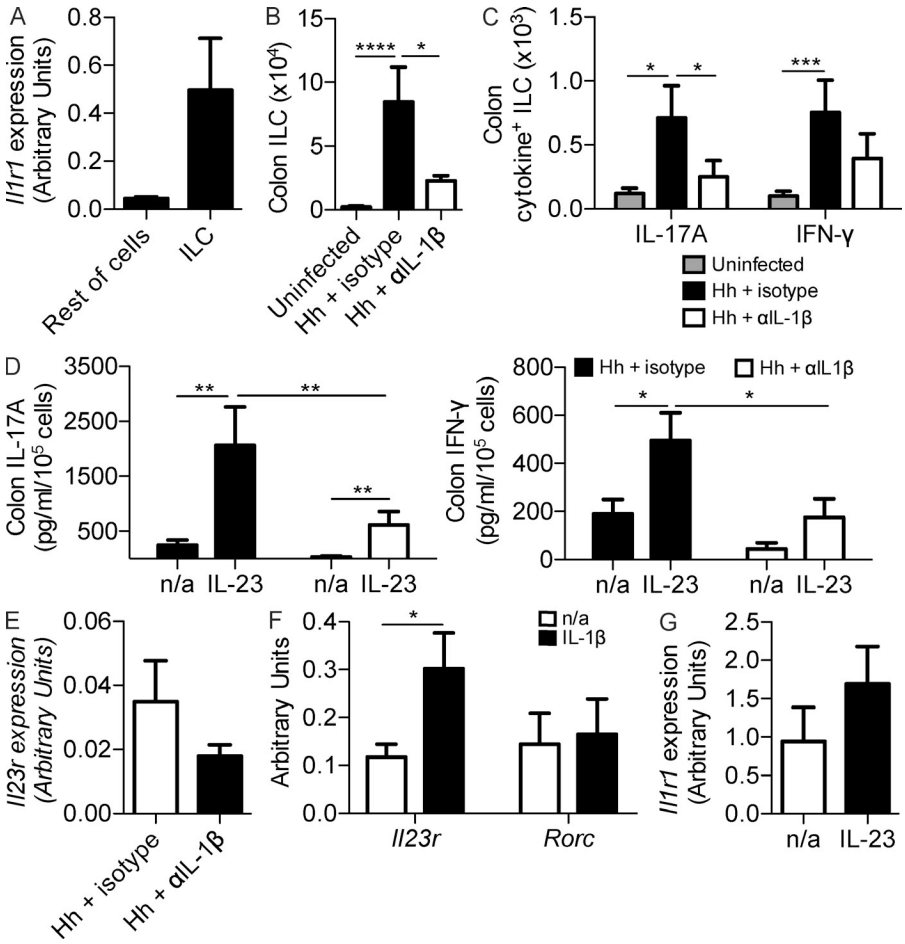

Figure 4. αIL-1β treatment reduces the accumulation of IL-17A–producing ILCs in the colon. (A) IL-1R1 expression by Sca1$^+$Thy1.2^{Hi} ILCs and rest of large intestine lamina propria cells. 129SvEv *Rag2*$^{-/-}$ mice were infected with *H. hepaticus* and sacrificed after >8 wk of infection. LPLs were isolated from the large intestine, Sca1$^+$Thy1.2^{Hi} ILCs were FACS sorted, and *Il1r1* expression was evaluated by qRT-PCR (mean ± SEM, $n = 2$ from 2 independent experiments; 8–10 mice were pooled in each experiment). (B–E) 129SvEv *Rag2*$^{-/-}$ mice were infected with *H. hepaticus* and treated weekly with 1 mg of αIL-1β antibody or isotype control (i.p.). After 8 wk, mice were sacrificed and cLPLs were isolated. (B) Total numbers of Sca1$^+$ Thy1.2^{Hi} ILCs in the colon lamina propria as evaluated by FACS analysis. (C) Total numbers of IL-17A– or IFN-γ–producing ILCs from the colon of indicated mice groups. (D) Cytokine production by cLPLs after overnight culture in complete medium alone (n/a) or in the presence of 10 ng/ml IL-23. Data are represented as mean ± SEM from 2 pooled independent experiments ($n = 6–11$). (E) *Il23r* expression by cLPLs as evaluated by qRT-PCR. Data are normalized on *Hprt* expression. (F and G) 129SvEv *Rag2*$^{-/-}$ mice were infected with *H. hepaticus* for 8 wk and CD45$^+$lin$^-$Sca1$^+$Thy1.2^{Hi} ILCs were FACS sorted from the colon. (F) ILCs were cultured overnight in complete medium alone (n/a) or in the presence of 10 ng/ml IL-1β. *Il23r* and *Rorc* expression levels were evaluated by qRT-PCR and normalized on *Hprt* expression. Data are shown as mean ± SEM from 2 pooled independent experiments. In each experiment, 10–18 mice were pooled. (G) ILCs were cultured overnight in complete medium alone (n/a) or in the presence of 10 ng/ml IL-23. *Il1r1* expression was evaluated by qRT-PCR and normalized on *Hprt* expression. Data are shown as mean ± SEM from two pooled independent experiments. In each experiment, 10–18 mice were pooled. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

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in rapid colitis and systemic cachexia (Powrie et al., 1993). Intestinal inflammation in this model of colitis was associated with increased levels of IL-1β secretion by cLPLs (Fig. 5 A). To assess the requirement for IL-1β stimulation of T cells in intestinal inflammation, we transferred naive $CD4^+CD45RB^{Hi}$ T cells isolated from either WT or *Il1r1*$^{-/-}$ mice into C57BL/6 *Rag1*$^{-/-}$ recipients and assessed the development of pathology. As expected, adoptively transferred WT naive $CD4^+$ T cells induced weight loss and severe intestinal and systemic inflammation in C57BL/6 *Rag1*$^{-/-}$ recipients (Fig. 5, B–F). In contrast, *Il1r1*$^{-/-}$ naive $CD4^+$ T cells failed to induce weight loss upon transfer to C57BL/6 *Rag1*$^{-/-}$ mice compared with recipients of WT naive $CD4^+$ T cells (Fig. 5 B). Moreover C57BL/6 *Rag1*$^{-/-}$ mice transferred with *Il1r1*$^{-/-}$ naive $CD4^+$ T cells showed significantly reduced intestinal pathology in both the cecum and the colon (Fig. 5, C–E). Despite attenuated intestinal inflammation, no significant difference was observed in either splenomegaly or spleen cellularity in C57BL/6 *Rag1*$^{-/-}$ mice receiving WT or *Il1r1*$^{-/-}$ naive $CD4^+$ T cells (Fig. 5 F). The attenuated intestinal inflammation in the colon of C57BL/6 *Rag1*$^{-/-}$ mice receiving *Il1r1*$^{-/-}$ naive $CD4^+$ T cells was accompanied by significant reductions in secretion of the proinflammatory cytokines IL-6, IFN-γ, TNF and IL-17A by cLPLs (Fig. 5 G), indicating a general dampening of the intestinal inflammatory response in the absence of IL-1R signaling on T cells.

IL-1R1 signaling promotes accumulation and survival of Th17 cells in the colon

We next analyzed the effects of cell-specific IL-1R signals on the accumulation of $CD4^+$ T cells in the colon. When compared with recipients of WT naive $CD4^+$ T cells, C57BL/6 *Rag1*$^{-/-}$ mice that received *Il1r1*$^{-/-}$ naive $CD4^+$ T cells exhibited a 50% decrease in the number of $CD4^+$ T cells present in the colon (Fig. 6 A), suggesting that IL-1R expression by T cells is essential to drive their accumulation in the intestine. Moreover, the effect of IL-1R signaling on accumulation of $CD4^+$ T cells was specific to the intestine, because similar $CD4^+$ T cell numbers were found in the MLN or spleen of C57BL/6 *Rag1*$^{-/-}$ mice receiving WT or *Il1r1*$^{-/-}$ naive $CD4^+$ T cells (Fig. 6 A).

The T cell transfer model of colitis is characterized by accumulation of Th1 and Th17 cells in the colon (Hue et al., 2006; Izcue et al., 2006). Recently, it has been proposed that

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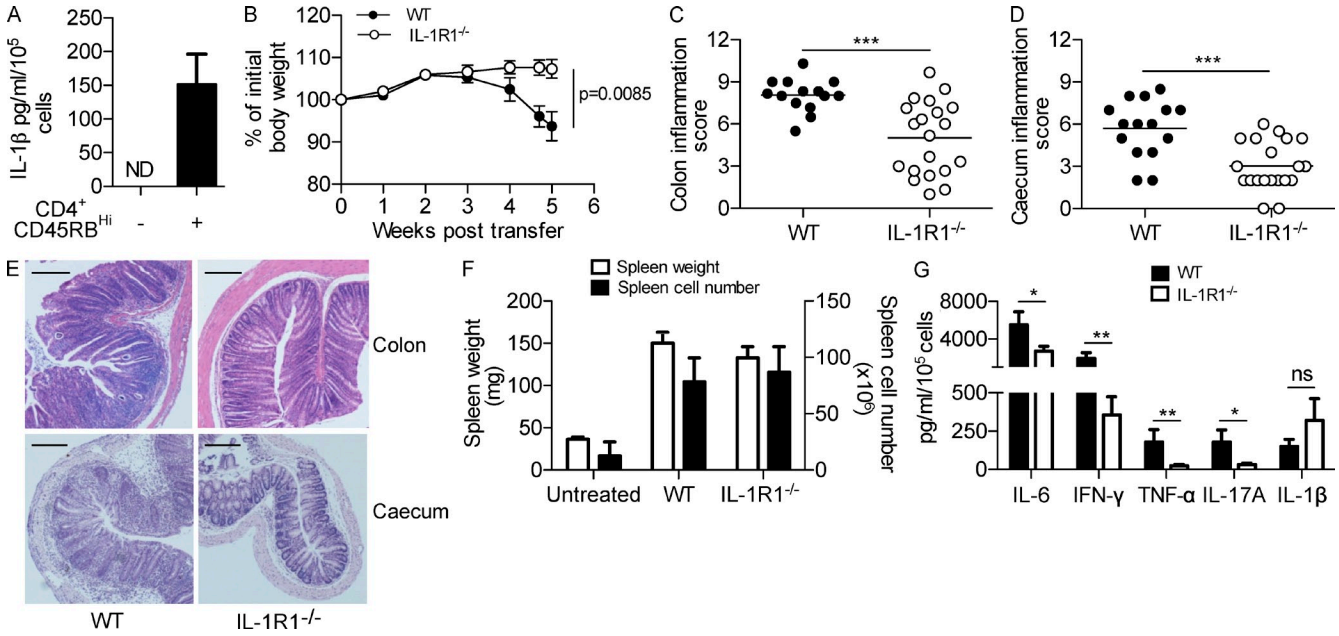

Figure 5. T cell–driven intestinal inflammation is attenuated in the absence of IL-1R signaling. (A) C57BL/6 *Rag1*$^{-/-}$ mice were transferred with 4×10^5 $CD4^+$ $CD25^-$ $CD45RB^{Hi}$ T cells from C57BL/6 WT mice and culled at the development of clinical signs of disease. cLPLs were isolated from the colon and cultured overnight in complete medium. IL-1β secretion in the supernatants was evaluated by FlowCytoMix kit (eBioscience; mean ± SEM; $n = 5$ for C57BL/6 *Rag1*$^{-/-}$ mice and $n = 10$ for recipients of $CD4^+CD45Rb^{Hi}$ T cells from 2 independent experiments). (B–G) C57BL/6 *Rag1*$^{-/-}$ mice were transferred with 4×10^5 $CD4^+$ $CD25^-$ $CD45RB^{Hi}$ T cells from C57BL/6 WT or *Il1r1*$^{-/-}$ mice. (B) Representative weight loss curve, shown as percentage of initial weight (data are shown as mean ± SEM; $n = 8$ mice/group, 1 representative of 3 independent experiments). (C and D) Intestinal inflammation scores for the colon (C) and the cecum (D) from three pooled independent experiments. Bars show the mean score. (E) Representative photomicrographs of colon and cecum (bar, 200 µm). (F) Spleen weight and spleen cell number. 1 representative experiment out of 3 is shown ($n = 3$ for untreated controls and $n = 8$ for naive $CD4^+$ T cell recipients). (G) Cytokine secretion by colonic lamina propria lymphocytes from C57BL/6 *Rag1*$^{-/-}$ mice transferred with WT or *Il1r1*$^{-/-}$ naive $CD4^+$ T cells. Data are shown as mean ± SEM ($n = 10$–14 from 2 pooled independent experiments). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ND not detectable.

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IL-1β plays a key role in promoting the differentiation of Th17 cells (Sutton et al., 2006; Chung et al., 2009; Sutton et al., 2009). In agreement with these findings, we found that after in vitro culture with TGF-β and IL-6, *Il1r1*$^{-/-}$ T cells exhibited decreased differentiation into Th17 cells when compared with WT CD4$^+$ T cells (Fig. 6 B). To assess the

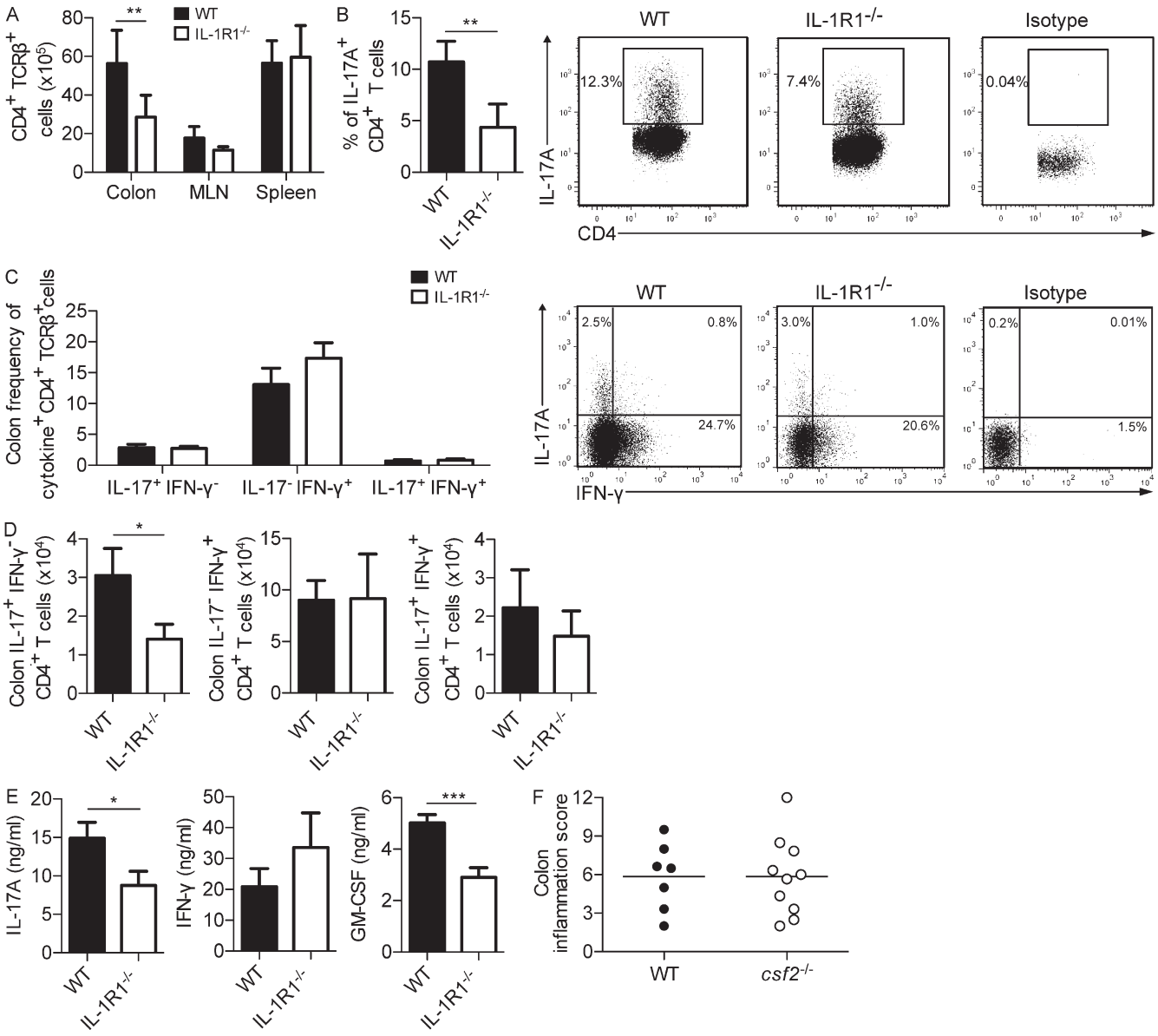

Figure 6. Impaired Th17 accumulation in the gut in the absence of IL-1R1 signaling. (A) C57BL/6 *Rag1*$^{-/-}$ mice were transferred with 4 × 10^5 CD4$^+$ CD25$^-$ CD45RB^{Hi} T cells from C57BL/6 WT or *Il1r1*$^{-/-}$ mice. At sacrifice, CD4$^+$ T cells in the colon, MLN and spleen were evaluated by FACS analysis. Data are shown as mean ± SEM (n = 20–21 from 3 pooled independent experiments). (B) Total CD4$^+$ T cells were isolated from the spleen of WT or *Il1r1*$^{-/-}$ mice and cultured in the presence of 2.5 ng/ml TGF-β and 50 ng/ml IL-6, plus plate-bound αCD3 (2 µg/ml) and αCD28 (1 µg/ml). After 3 d, the frequency of IL-17A–producing cells was analyzed by intracellular staining. Data are shown as mean ± SD from 2 pooled independent experiments (n = 4), together with representative FACS plots (gated on CD4$^+$TCRβ$^+$ cells). (C–E) C57BL/6 *Rag1*$^{-/-}$ mice were transferred with 4 × 10^5 CD4$^+$ CD25$^-$ CD45RB^{Hi} T cells from C57BL/6 WT or *Il1r1*$^{-/-}$ mice. (C and D) At sacrifice, colonic lamina propria leukocytes were isolated and analyzed by intracellular FACS analysis. (C) Frequency of indicated T helper subsets among CD4$^+$ T cells. In the representative FACS plot shown on the right, data are gated on CD4$^+$TCRβ$^+$ cells. (D) Accumulation of effector CD4$^+$ T cell populations in the colon. Data are shown as mean ± SEM (n = 20–21 from 3 pooled independent experiments). (E) CD4$^+$ T cells were FACS sorted from the colon of recipients of *Il1r1*$^{-/-}$ or WT naive CD4$^+$ T cells and restimulated overnight with PMA and ionomycin. Cytokine secretion in the supernatants was assessed by FlowCytomix (n = 12–14 from 3 pooled independent experiments). (F) C57BL/6 *Rag1*$^{-/-}$ mice were transferred with 4 × 10^5 CD4$^+$ CD25$^-$ CD45RB^{Hi} T cells from C57BL/6 WT or *csf2*$^{-/-}$ mice and culled at development of clinical signs of disease. Colonic inflammation score was assessed histologically (n = 7–10). *, P < 0.05; **, P < 0.01; ***, P < 0.001.

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role of IL-1R expression on T helper cell differentiation in vivo, we isolated CD4$^+$ T cells from the colon of C57BL/6 *Rag1*$^{-/-}$ mice receiving WT or *Il1r1*$^{-/-}$ naive CD4$^+$ T cells and performed intracellular staining for IL-17A and IFN-γ production. We found that IL-1R expression was dispensable for Th1 and Th17 cell differentiation in the colon, as shown by similar frequencies of these effector cells observed in recipients of WT or *Il1r1*$^{-/-}$ naive CD4$^+$ T cells (Fig. 6 C). IL-1R expression was also dispensable for Th17 differentiation in the MLN (not depicted). Furthermore, we observed similar numbers of CD4$^+$ IL-17A$^-$IFN-γ$^+$ Th1 cells and IL-17A$^+$IFN-γ$^+$ T cells in the colon of C57BL/6 *Rag1*$^{-/-}$ mice receiving *Il1r1*$^{-/-}$ or WT naive CD4$^+$ T cells (Fig. 6 D). In contrast, we observed a specific defect in the accumulation of IL-17A–producing CD4$^+$ T cells, as shown by a 50% decrease in the number of IL-17A$^+$ IFN-γ$^-$ T cells found in the colon of C57BL/6 *Rag1*$^{-/-}$ mice receiving *Il1r1*$^{-/-}$ naive CD4$^+$ T cells when compared with recipients of WT naive CD4$^+$ T cells (Fig. 6 D). To further examine the role of IL-1R1 signaling on T cell effector functions, we isolated CD4$^+$ T cells from the colon of mice transferred either with WT or *Il1r1*$^{-/-}$ naive CD4$^+$ T cells and examined their cytokine profile ex vivo. In accordance with our previous data, colonic *Il1r1*$^{-/-}$ CD4$^+$ T cells secreted less IL-17A, whereas the levels of IFN-γ secretion were unaffected (Fig. 6 E). Together, these data indicate a role for T cell–specific IL-1R1 signaling in promoting the accumulation of CD4$^+$ T cells in the colon, with a specific defect in Th17 cells.

Recent reports show that IL-1β promotes GM-CSF production by Th17 cells, and that GM-CSF plays a key role in development of experimental autoimmune encephalomyelitis (EAE; Codarri et al., 2011; El-Behi et al., 2011). We found that colonic *Il1r1*$^{-/-}$ CD4$^+$ T cells isolated after T cell transfer secreted significantly less GM-CSF than colonic WT CD4$^+$ T cells (Fig. 6 E), prompting us to assess the role of T cell–derived GM-CSF in promoting intestinal inflammation. To this purpose, we adoptively transferred GM-CSF–deficient (*csf2*$^{-/-}$) naive CD4$^+$CD45RB^{Hi} T cells to C57BL/6 *Rag1*$^{-/-}$ mice. We observed that the level of colonic inflammation was similar between mice receiving WT or *csf2*$^{-/-}$ naive CD4$^+$ T cells (Fig. 6 F). Cecal inflammation and splenomegaly were also unaffected by the inability of naive T cells to produce GM-CSF, and the proportions and numbers of IL-17A$^+$IFN-γ$^-$, IL-17A$^+$IFN-γ$^+$ and IL-17A$^-$IFN-γ$^+$ effector CD4$^+$ T cells were similar in recipients of *csf2*$^{-/-}$ and WT naive T cells (unpublished data). These data indicate that T cell–derived GM-CSF is not required to induce intestinal inflammation or to facilitate Th17 cell differentiation in the gut.

Next, we examined whether the defective accumulation of Th17 cells in the absence of cell-specific IL-1R expression could be caused by defective proliferation of the *Il1r1*$^{-/-}$ T cells in the colon. However, CD4$^+$ T cells and Th17 cells isolated from the colon of C57BL/6 *Rag1*$^{-/-}$ mice receiving *Il1r1*$^{-/-}$ or WT naive CD4$^+$ T cells expressed the proliferation marker Ki67 to a similar extent (Fig. 7 A). Moreover, adoptive transfer of CFSE-labeled *Il1r1*$^{-/-}$ or WT naive T cells revealed similar CFSE dilution profiles 12 d after transfer (unpublished data), suggesting that *Il1r1*$^{-/-}$ T cells undergo comparable early expansion after adoptive transfer. Alternatively, we hypothesized that the absence of IL-1R signals could compromise the ability of CD4$^+$ T cells to migrate to the colon. To test this hypothesis, we transferred *Il1r1*$^{-/-}$ or WT CD4$^+$CD45RB^{Hi} T cells into C57BL/6 *Rag1*$^{-/-}$ mice and sacrificed them 2 wk after transfer, before the onset of colitis. At this early time point, no difference in the total number of CD4$^+$ T cells or Th17 cells present in the colon was observed between C57BL/6 *Rag1*$^{-/-}$ mice receiving *Il1r1*$^{-/-}$ or WT naive CD4$^+$ T cells (Fig. 7 B). Moreover, Ki67 expression by total CD4$^+$ T cells or by Th17 cells was again comparable in WT and *Il1r1*$^{-/-}$ CD4$^+$ T cells (unpublished data). In addition, *Il1r1*$^{-/-}$ CD4$^+$ T cells exhibited comparable expression of the chemokine receptors *Ccr6, Ccr2, Ccr5*, and *Cxcr6* (Fig. 7 C). Together, these results excluded an effect of IL-1R expression in preventing T cell migration to the colon or in impairing early T cell proliferation.

We therefore hypothesized that the defective accumulation of naive *Il1r1*$^{-/-}$ CD4$^+$ T cells could be caused by impaired survival. To assess this point, we first tried to quantify apoptotic T cells in vivo by staining colonic sections from colitic mice by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) or caspase-3 staining combined with αCD3 immunofluorescence staining. However, the frequency of CD3$^+$ apoptotic cells in these assays was too low to allow accurate quantification of apoptotic events, probably reflecting the rapid clearance of apoptotic cells in vivo (unpublished data). Therefore, we FACS sorted CD4$^+$ T cells from the colon of C57BL/6 *Rag1*$^{-/-}$ mice receiving either WT or *Il1r1*$^{-/-}$ naive CD4$^+$ T cells and assessed the expression of the antiapoptotic factors *Bcl2* and *Bclxl* by qRT-PCR. Our analysis showed that colonic CD4$^+$ T cells from mice receiving *Il1r1*$^{-/-}$ naive CD4$^+$ T cells expressed significantly lower levels of *Bcl2* and *Bclxl* compared with those from mice transferred with WT naive CD4$^+$ T cells (Fig. 7 D). Furthermore, *Il1r1*$^{-/-}$ CD4$^+$ T cells exhibited significantly higher levels of apoptosis than WT CD4$^+$ T cells after stimulation with αCD3 and αCD28 mAb in vitro (Fig. 7 E), supporting the hypothesis that IL-1R1 signaling promotes T cell survival during inflammation, by preventing activation-induced apoptosis. In contrast, stimulation of freshly isolated ILCs with IL-1β did not affect their expression of *Bcl2* (Fig. 7 F).

T cell–specific IL-23R signals are also required for intestinal inflammation, as *Il23r*$^{-/-}$ naive CD4$^+$ T cells failed to induce intestinal pathology upon T cell transfer to C57BL/6 Rag$^{-/-}$ mice (Ahern et al., 2010). Initially, we hypothesized that, similar to ILCs, T cells required *Il1r1* signals to upregulate *Il23r* expression. However, FACS sorted CD4$^+$ T cells from the colon of mice transferred with either WT or *Il1r1*$^{-/-}$ naive CD4$^+$ T cells expressed similar levels of IL-23R (Fig. 8 A). Because it has been reported that IL-1R1 expression is upregulated in Th17 cells (Chung et al., 2009), we conjectured that in the colon this might be driven by IL-23, which is key for the generation of colitogenic CD4$^+$ T cell responses

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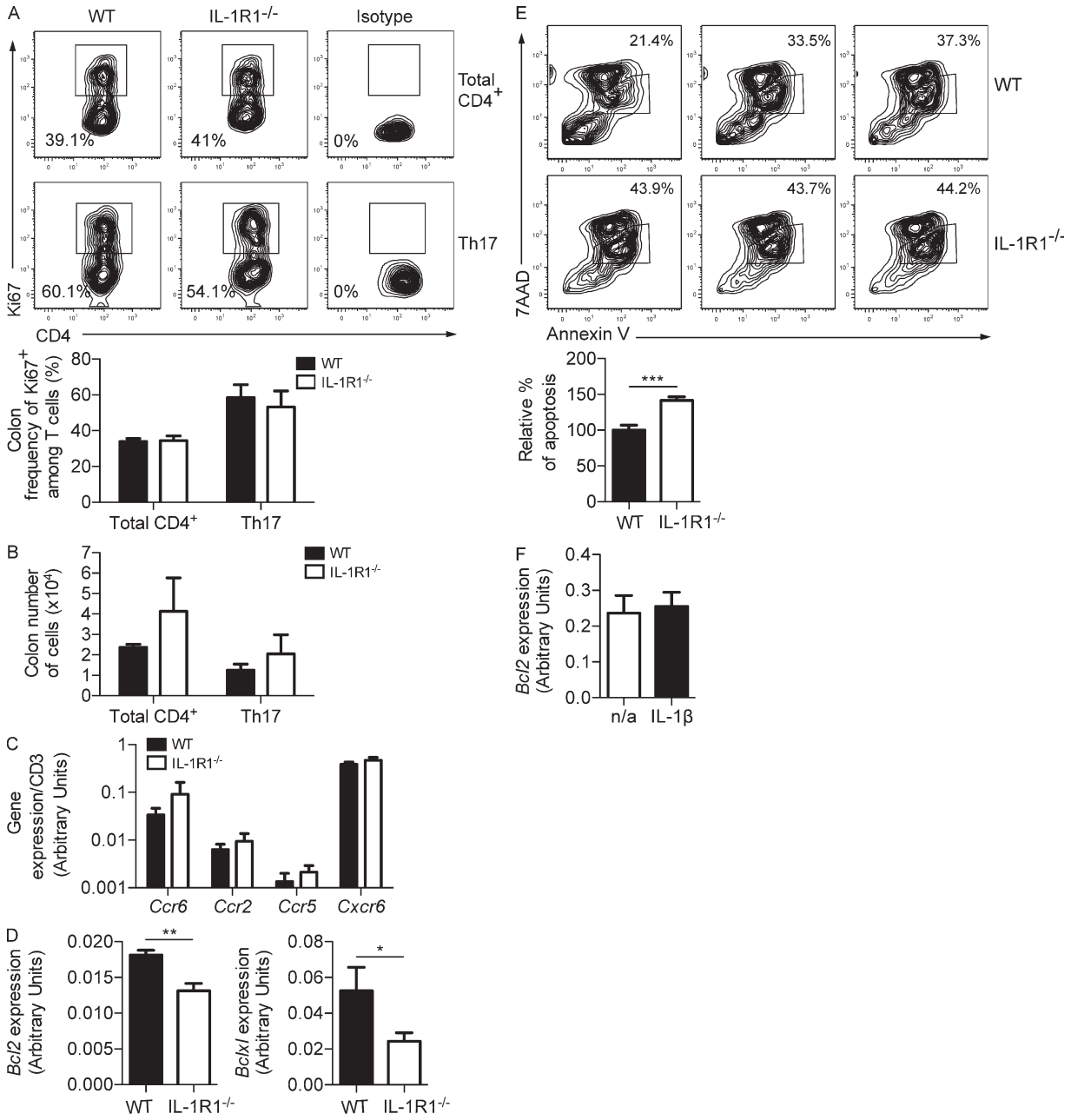

Figure 7. IL-1R signaling modulates CD4⁺ T cell apoptosis in vivo and in vitro. (A) C57BL/6 *Rag1*$^{-/-}$ mice were transferred with 4×10^5 CD4⁺ CD25⁻ CD45RB^{Hi} T cells from C57BL/6 WT or *Il1r1*$^{-/-}$ mice. At sacrifice, cLPLs were isolated and Ki67 expression by cLPLs CD4⁺ T cells was assessed by intracellular staining. Data represent mean ± SEM (n = 20–21 from 3 pooled independent experiments). Representative FACS plots (gated on CD4⁺ T cells) and quantitation are shown. (B) C57BL/6 *Rag1*$^{-/-}$ mice were transferred with 2×10^6 CD4⁺ CD25⁻ CD45Rb^{Hi} T cells from C57BL/6 WT or *Il1r1*$^{-/-}$ mice and sacrificed 2 wk after transfer. Total number of CD4⁺ T cells and Th17 cells from the colonic lamina propria were assessed by FACS. Data are shown as mean ± SEM (n = 5 mice/group). (C and D) C57BL/6 *Rag1*$^{-/-}$ mice were transferred with 4×10^5 CD4⁺ CD25⁻ CD45RB^{Hi} T cells from C57BL/6 WT or *Il1r1*$^{-/-}$ mice. Mice were sacrificed when recipients of WT naive CD4⁺ T cells developed clinical signs of colitis, and CD4⁺ T cells were FACS sorted from cLPL preparations. (C) Expression levels of indicated chemokine receptors as revealed by qRT-PCR analysis. (D) Bcl-2 and Bcl-XL expression by WT or *Il1r1*$^{-/-}$ CD4⁺ T cells upon ex vivo restimulation with 0.1 µg/ml PMA and 1 µg/ml ionomycin overnight in complete medium (n = 5 mice/group, 1 representative experiment out of 2 is shown). (E) Purified CD4⁺ T cells from WT or *Il1r1*$^{-/-}$ mice were cultured for 3 d in the presence of plate-bound αCD3 and αCD28 mAb at the indicated concentrations. After staining with the phosphatidylserine-binding protein Annexin V and the vital dye 7AAD, Annexin V⁺ 7AAD⁻ cells were identified as early apoptotic events. The average frequency of Annexin V⁺ 7AAD⁻ cells in WT cells was set as a baseline (100%), and data were expressed as relative increase over baseline (n = 10 from 4 pooled independent experiments). Representative FACS plots (gated on CD4⁺ T cells) and quantitation are shown. (F) 129SvEv Rag2$^{-/-}$ mice were infected with *H. hepaticus* for 8 wk. CD45⁺lin⁻ Sca1⁺Thy1.2^{Hi} ILCs were FACS sorted from the colon and cultured overnight in complete medium alone (n/a) or in the presence of IL-1β (10ng/ml). Bcl2 expression was evaluated by qRT-PCR and normalized on *Hprt* expression. Data are shown as mean ± SEM from two pooled independent experiments. In each experiment, 10–18 mice were pooled.

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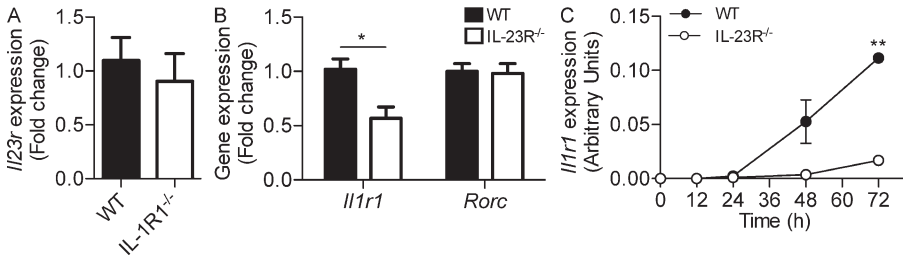

Figure 8. IL-1R1 expression by colonic CD4+ T cells is dependent on IL-23 stimulation. (A) C57BL/6 *Rag1*$^{-/-}$ mice were transferred with 4 × 10^5 CD4$^+$ CD25$^-$ CD45RB^{Hi} T cells from C57BL/6 WT or *Il1r1*$^{-/-}$ mice. Mice were sacrificed when recipient of WT naive CD4$^+$ T cells developed clinical signs of colitis. CD4$^+$ T cells were FACS sorted from cLPL preparations and *Il23r* expression was evaluated by qRT-PCR (n = 5 mice/group, 1 out of 2 representative experiments shown). (B) C57BL/6 mice were transferred with 1:1 mixtures of CD45.1$^+$ (WT) and CD45.2$^+$ (*Il23r*$^{-/-}$) CD4$^+$CD45RB^{hi} T cells. Mice were sacrificed upon development of clinical signs of inflammation (~6 wk) and WT or *Il23r*$^{-/-}$ populations of T cells were FACS sorted based on the expression of CD45.2. *Il1r1* and *Rorc* expression levels were evaluated by qRT-PCR (data are shown as mean ± SEM; n = 6 from 2 independent experiments). (C) CD62L^{Hi} CD44^{low} CD25$^-$ naive CD4$^+$ T cells isolated from WT or *Il23r*$^{-/-}$ mice were cultured under Th17-polarizing conditions. At the indicated time points, *Il1r1* expression was evaluated by qRT-PCR (data are shown as mean ± SD; n = 2 from 2 pooled independent experiments). *, $P < 0.05$; **, $P < 0.01$.

(Ahern et al., 2010). To test this hypothesis, we co-transferred equal numbers of CD45.1$^+$ WT and CD45.2$^+$ *Il23r*$^{-/-}$ naive CD4$^+$ T cells into C57BL/6 Rag$^{-/-}$ recipients and, after the development of colitis, we isolated colonic WT and *Il23r*$^{-/-}$ CD4$^+$ T cells by FACS sorting based on the expression of CD45.2. This strategy allows comparison of *Il23r*$^{-/-}$ and WT CD4$^+$ T cells that have been conditioned in an identical inflamed intestinal environment (Ahern et al., 2010). Analysis by qRT-PCR revealed that colonic *Il23r*$^{-/-}$ CD4$^+$ T cells expressed significantly lower levels of *IL1r1* than colonic WT CD4$^+$ T cells (Fig. 8 B), suggesting that T cell–specific IL-23R signals promoted increased sensitivity to IL-1 in vivo. However, the levels of expression of the Th17 signature transcription factor *Rorc* were similar between the two groups, suggesting that the observed reduction of IL-1R expression is not caused by a general impairment of Th17 differentiation in vivo (Fig. 8 B). Consistent with this hypothesis, *Il23r*$^{-/-}$ naive CD4$^+$ T cells failed to up-regulate *IL1r1* when cultured under Th17 polarizing conditions (including IL-23) in vitro, whereas WT naive CD4$^+$ T cells demonstrated a marked increase in *IL1r1* expression over the culture period (Fig. 8 C). Together, these data suggest that IL-23 signaling on T cells promotes IL-1R expression, thereby enhancing T cell sensitivity to IL-1.

DISCUSSION

Numerous studies link intestinal inflammation in IBD patients with increased intestinal levels of IL-1β. However, a lack of preclinical research into the role of IL-1β in promoting chronic intestinal inflammation has hampered the translation of these clinical observations into potential therapeutic strategies, in spite of new evidence in humans linking dysregulated IL-1β secretion with polymorphisms associated to IBD development (Plantinga et al., 2011). Here, using two complementary models of intestinal inflammation, we have dissected the cellular mechanisms through which IL-1β contributes to intestinal pathology. We found that IL-1β was essential for the development of severe innate immune pathology driven by the intestinal pathogen *H. hepaticus*. We identified an intestine-specific role for IL-1β in driving classical pathways of innate immune inflammation, by promoting the recruitment of granulocytes to the colon. In addition, we showed that IL-1β stimulation of CD4$^+$ T cells was required for the induction of intestinal inflammation in a T cell transfer model of colitis by promoting the accumulation and survival of CD4$^+$ T cells in the colon. Moreover, we observed a crucial role for IL-1β in inducing "type-17" responses from both innate and adaptive leukocytes in the colon.

Using a T cell–independent model of colitis, we assessed the role of IL-1β in driving innate intestinal inflammation. In accordance with the central role played by IL-1β in orchestrating innate inflammatory responses (Dinarello, 1996; Sims and Smith, 2010), we observed attenuation of *H. hepaticus*–induced colitis after αIL-1β treatment. The common adaptor protein, MyD88, is responsible for signaling downstream of the IL-1R (Dinarello, 2009) and previous studies from our laboratory showed *MyD88*$^{-/-}$ mice to be completely protected from *H. hepaticus*–induced colitis (Asquith et al., 2010). Interestingly, *H. hepaticus*–infected *MyD88*$^{-/-}$ mice were completely protected from typhlocolitis, whereas only colitis was significantly decreased in αIL-1β–treated mice, implying that IL-1β contributes mainly to proinflammatory MyD88-dependent signals in the colon. Consistent with this hypothesis, *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice treated with αIL-1β exhibited a selective decrease in neutrophil recruitment in the colon. Others have reported that blocking the IL-1β pathway ameliorated DSS-triggered intestinal inflammation (Thomas et al., 1991; Cominelli et al., 1992; Siegmund et al., 2001). IL-1R deficiency in these models did not perturb granulocyte recruitment to the colon. However, DSS colitis represents an acute model of colitis where granulocyte recruitment is secondary to the extensive epithelial damage caused by the initial bacterial or chemical injury. In contrast, *H. hepaticus*–induced intestinal pathology in 129SvEv *Rag2*$^{-/-}$ mice is primarily associated with chronic activation of innate leukocytes in the lamina propria (Asquith and Powrie, 2010; Asquith et al., 2010). Hence the mechanisms underlying intestinal pathology and leukocyte recruitment are profoundly different between these models of colitis.

Our data show that IL-1β acts to boost IL-17A production in the intestine of *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice.

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Given that IL-17A production contributes to pathology in this model (Buonocore et al., 2010), it is possible that the decreased inflammation observed after treatment with αIL-1β is due, at least in part, to its effects on IL-17A. We further observed that IL-1β was required for the accumulation of a novel population of IL-17A–producing ILCs, which express high levels of IL-1R1. Fewer numbers of Sca1$^+$ Thy1.2^{Hi} ILCs were present in the colon of *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice receiving αIL-1β compared with those treated with an isotype control. In addition, ILCs isolated from the colon of *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice that were treated with αIL-1β had reduced frequencies of IL-17A– and IFN-γ–secreting cells, which correlated with decreased secretion of these cytokines in response to IL-23 stimulation. Thus, IL-1β signals promote the accumulation and effector functions of ILCs in the intestine. A role for IL-1β in promoting the in vitro proliferation and function of a distinct innate lymphoid cell type has been proposed. NK-22 or RORγt$^+$CD127$^+$ NK-like cells are present at mucosal sites and can secrete both IL-22 and IL-17A in response to IL-23 (Cella et al., 2009; Cupedo et al., 2009). Recently, it has been shown that IL-1β can strongly synergize with other cytokines, such as IL-7, IL-15, or IL-23, to promote proliferation and effector cytokine secretion by NK-22 cells in vitro (Cella et al., 2010; Hughes et al., 2010; Reynders et al., 2011). Thus, IL-1β may play a crucial role in modulating the functions of several types of innate lymphoid cells at mucosal sites. The exact mechanisms underlying these effects remain unclear, but it is possible that IL-1β acts in concert with other factors to regulate ILC function, as IL-1β stimulation alone has only minor effects on innate lymphoid cell biology (Cupedo et al., 2009; Hughes et al., 2010). In support of this hypothesis, we found that ILCs cultured with IL-1β had significantly higher levels of *Il23r* expression than those cultured with medium alone, suggesting that IL-1R1 signals maintain or enhance IL-23 responsiveness in ILCs.

The ability of IL-1β to promote accumulation of immune effector cells in the intestine was paralleled in our studies using a T cell–dependent colitis model. The reduced ability of *Il1r1*$^{-/-}$ naive CD4$^+$ T cells to induce severe colitis upon transfer to C57BL/6 *Rag1*$^{-/-}$ recipients was associated with reduced accumulation of CD4$^+$ T cells. Remarkably, the accumulation defect was restricted to the intestine, with similar numbers of CD4$^+$ T cells found in the spleen or MLN in recipients of *Il1r1*$^{-/-}$ CD4$^+$ T cells. This would suggest that although not required for the reconstitution of lymphopenic mice, T cell–specific IL-1R signaling is a prerequisite for T cell accumulation in the intestine. Mechanistic analysis revealed that this effect was not caused by defective proliferation or migration of *Il1r1*$^{-/-}$ T cells, suggesting a role of IL-1β in promoting T cell survival. This hypothesis was confirmed by the observation that the expression of the antiapoptotic proteins Bcl-2 and Bcl-xL was impaired in colonic *Il1r1*$^{-/-}$ CD4$^+$ T cells after transfer to lymphopenic mice. These results are consistent with previous findings that naive *MyD88*$^{-/-}$ CD4$^+$ T cells also exhibited impaired ability to accumulate in the intestine and mediate colitis after transfer into C57BL/6 *Rag1*$^{-/-}$ mice, associated with reduced expression of Bcl2 and Bcl-xL (Fukata et al., 2008; Tomita et al., 2008; Asquith et al., 2010). Overall, these data suggest that IL-1R/Myd88 signaling is crucial to promote T cell survival during intestinal inflammation.

Defective CD4$^+$ T cell accumulation in the intestine after transfer of naive *Il1r1*$^{-/-}$ CD4$^+$ T cells was primarily caused by decreased numbers of Th17 cells. Previous studies in humans and mice have identified a role for IL-1 in promoting Th17 differentiation, and autoinflammatory diseases characterized by high levels of IL-1β are associated with a marked Th17 signature at the effector site (Horai et al., 2000; Aksentijevich et al., 2009; Brydges et al., 2009; Meng et al., 2009; Reddy et al., 2009; Lamacchia et al., 2010). Additionally, in vitro studies demonstrated a clear role for IL-1β, in conjunction with the proinflammatory cytokines IL-6 and IL-23, in promoting the differentiation of Th17 cells (Sutton et al., 2006, 2009; Chung et al., 2009; Ghoreschi et al., 2010), and *Il1r1*$^{-/-}$ mice show defective Th17 differentiation in the small intestine at steady state (Shaw et al., 2012). Furthermore, T cell–specific IL-1R expression is necessary for the induction of EAE in vivo, as adoptive transfer of *Il1r1*$^{-/-}$ T cells resulted in decreased frequencies of Th17 cells in the central nervous system (Sutton et al., 2006; Chung et al., 2009). Our in vitro studies confirmed the requirement for IL-1R expression in Th17 differentiation. However, comparable frequencies of IL-17–producing CD4$^+$ T cells were found in the colon of C57BL/6 *Rag1*$^{-/-}$ mice receiving WT or *Il1r1*$^{-/-}$ naive CD4$^+$ T cells, which may reflect the highly polarizing Th17 microenvironment in the colon (Atarashi et al., 2008; Ivanov et al., 2009). Although dispensable for Th17 differentiation in the gut upon naive T cell transfer, IL-1R signaling was necessary to promote Th17 accumulation, as shown by the decreased numbers of Th17 cells found in the colon of C57BL/6 *Rag1*$^{-/-}$ mice receiving *Il1r1*$^{-/-}$ naive CD4$^+$ T cells. This effect was specific to Th17 cells and was not observed for IL-17A$^-$ IFN-γ$^+$ or IL-17A$^+$ IFN-γ$^+$ CD4$^+$ T cells, showing that *Il1r1*$^{-/-}$ T cells are not generally impaired in their proinflammatory capacity. This could be explained by high levels of expression of *Il1r1* by Th17 cells, which may render them more sensitive to, or dependent on, IL-1R stimulation (Chung et al., 2009; Guo et al., 2009). Because proliferation and migration to the gut of *Il1r1*$^{-/-}$ naive CD4$^+$ T cells was unimpaired, it is possible that the main function for IL-1R signals in modulating Th17 biology in the gut is to promote their survival during inflammation. This hypothesis is consistent with other studies showing that IL-1β can promote Th17 expansion and cytokine production in vitro in the absence of TCR stimulation (Sutton et al., 2006), suggesting a role of IL-1β in homeostatic maintenance of Th17 cells.

We also present evidence of synergy between IL-23R and IL-1R signals for the induction and maintenance of pathogenic Th17 responses. A previous in vitro study reported that IL-1R signaling only modestly enhanced *Il23r* expression by

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$CD4^+$ T cells (Chung et al., 2009). Similarly, we found that colonic $CD4^+$ T cells isolated from C57BL/6 *Rag1*$^{-/-}$ mice that received either WT or *Il1r1*$^{-/-}$ naive $CD4^+$ T cells expressed comparable levels of *Il23r*. In contrast, when $CD4^+$ T cells were isolated from the inflamed colon of C57BL/6 *Rag1*$^{-/-}$ mice that were co-transferred with both WT and *Il23r*$^{-/-}$ naive $CD4^+$ T cells, we found that the *Il23r*$^{-/-}$ $CD4^+$ T cells expressed significantly lower levels of *Il1r1*. Furthermore, *Il23r*$^{-/-}$ naive $CD4^+$ T cells failed to increase expression of *Il1r1* during culture in IL-23–supplemented, Th17-polarizing conditions in vitro. Together, these observations suggest that IL-23R signals play a key role in the up-regulation and maintenance of IL-1R expression by Th17 cells. As IL-1R signals promote T cell survival, this process establishes a positive feedback loop promoting T cell accumulation in the colon.

Recent evidence suggests that IL-1β promotes GM-CSF production by Th17 cells and that T cell–derived GM-CSF plays a key role in EAE pathology (Codarri et al., 2011; El-Behi et al., 2011). Although we found that colonic $CD4^+$ T cells isolated from C57BL/6 *Rag1*$^{-/-}$ mice receiving *Il1r1*$^{-/-}$ $CD4^+$ naive T cells showed reduced GM-CSF production, adoptive transfer of GM-CSF–deficient $CD4^+$ naive T cells resulted in intestinal inflammation with similar features to that observed in C57BL/6 *Rag1*$^{-/-}$ mice receiving WT $CD4^+$ T cells. Hence, although necessary for the development of EAE, T cell–derived GM-CSF is not required for the induction of colitis.

In summary, our data clearly show an effect of blocking IL-1β in ameliorating chronic intestinal inflammation, as well as providing mechanistic insight into the proinflammatory actions of IL-1β in the intestine. This study also highlights the synergistic activities of IL-1β and IL-23 in promoting pathogenic innate and adaptive type 17 responses in the intestine. This hypothesis is supported by the finding that IL-1β and IL-23 are expressed with similar kinetics during the development of intestinal inflammation ((Boulard et al., 2012) and unpublished data) and by previous studies showing that IL-1β and IL-23 promote a Th17-like phenotype in both $CD4^+$ T cells and NK-22 cells in vitro (Chung et al., 2009; Cella et al., 2010; Ghoreschi et al., 2010). Numerous IL-1β–blocking reagents have been developed for clinical studies, many of which have proven successful in treating inflammatory disorders (Gabay et al., 2010). Moreover, recent data show exacerbated IL-1β production upon NOD2 stimulation of PBMCs from IBD patients bearing a risk variant of the *ATG16L1* gene (Plantinga et al., 2011), confirming that IL-1β is a central effector mechanism downstream of different IBD-related inflammatory pathways. Considering the profound dysregulation of the IL-1 pathway observed in the gut of IBD patients, targeting this pathway may represent a future therapeutic approach in IBD.

MATERIALS AND METHODS

Mice. 129SvEv *Rag*$^{-/-}$, C57BL/6, C57BL/6 *Rag1*$^{-/-}$, C57BL/6 *Il1r1*$^{-/-}$, and *Il23r*$^{-/-}$ C57BL/6 strains were bred and maintained under specific pathogen–free conditions in accredited animal facilities at the University of Oxford. All experiments were performed in accordance with the UK Scientific Procedures Act (1986) under a Project License (PPL) authorized by the UK Home Office Animal Procedures Committee and approved by the Sir William Dunn School of Pathology Local Ethical Review Committee. C57BL/6 *csf2*$^{-/-}$ mice were provided by B. Becher (University of Zurich, Zurich, Switzerland). Mice were routinely screened for *Helicobacter spp*. and were >6 wk of age when used.

Bacteria. *H. hepaticus* NCI-Frederick isolate 1A, isolated from the same mouse colony as isolate Hh-1 (strain 51449; American Type Culture Collection), was grown on blood agar plates containing Trimethoprim, vancomycin, and polymixin B (Oxoid) under microaerophilic conditions, as previously described (Maloy et al., 2003; Young et al., 2004). *H. hepaticus* viability was confirmed using fluorescent microscopy with a bacterial live/dead kit (BacLight; Invitrogen). For induction of innate immune colitis, 129SvEv *Rag2*$^{-/-}$ mice were infected with *H. hepaticus* (~10^8 CFU) by oral gavage three times on alternate days. *H. hepaticus*–infected mice were sacrificed 8–10 wk after infection.

In vivo antibody treatment. To block IL-1β activity in vivo, *H. hepaticus*–infected 129SvEv *Rag2*$^{-/-}$ mice were injected i.p. with 1 mg αIL-1β–blocking mAb (αIL-1β) or 1 mg isotype control mAb from the day of the first inoculation with *H. hepaticus*, and treatment repeated weekly for the duration of the experiment.

Induction of colitis with naive $CD4^+CD45RB^{Hi}$ T Cells. Naive $CD4^+CD45RB^{Hi}$ T cells were purified (>98%) from spleens of C57BL/6, C57BL/6 *Il1r1*$^{-/-}$, *IL23r*$^{-/-}$, or *Csf2*$^{-/-}$ mice via FACS sorting, as previously described (Izcue et al., 2008). In brief, single-cell suspensions were depleted of $CD8^+$, MHC class II^+, $Mac\text{-}1^+$, and $B220^+$ cells by negative selection using a panel of rat monoclonal antibodies, followed by sheep anti–rat–coated Dynabeads (Invitrogen). After staining with APC-conjugated αCD4, PE-conjugated αCD25, and FITC–αCD45RB, naive $CD4^+CD45RB^{Hi}$ T cells were purified by cell sorting with a cell sorter (MoFlo; Dako). Naive T cell suspensions were washed in sterile PBS, and age- and sex-matched C57BL/6 *Rag1*$^{-/-}$ recipient mice received 4×10^5 $CD4^+CD45RB^{Hi}$ T cells by i.p. injection. For co-transfer experiments, 1:1 mixtures of $CD45.2^+$ *Il23r*$^{-/-}$ and $CD45.1^+$ WT $CD4^+CD45RB^{Hi}$ T cells were injected i.p. (total cell number = 4×10^5). Mice were sacrificed when symptoms of clinical disease (weight loss and/or diarrhea) developed in control groups, ~5–8 wk after transfer of naive $CD4^+$ T cells, unless otherwise indicated. For mice sacrificed 2 wk after transfer, 2×10^6 $CD4^+CD45RB^{Hi}$ T cells were transferred per mouse.

Assessment of intestinal inflammation. Samples of cecum and proximal, mid, and distal colon were prepared as previously described (Izcue et al., 2008), and inflammation was graded according to the following scoring system. Each sample was graded semiquantitatively from zero to three for four criteria: (1) degree of epithelial hyperplasia and goblet cell depletion; (2) leukocyte infiltration in the lamina propria; (3) area of tissue affected; and (4) the presence of markers of severe inflammation such as crypt abscesses, submucosal inflammation, and ulcers. Scores for each criterion were added to give an overall inflammation score for each sample of 0–12. Scores from proximal, mid, and distal colon were averaged to obtain inflammation scores for the colon. Sections were scored in a blinded fashion to avoid bias.

Isolation of leukocyte subpopulations and FACS. Cell suspensions from spleen, liver, MLN, and the lamina propria were prepared as described previously (Buonocore et al., 2010). Cells were washed, incubated with anti-Fc receptor (αCD16/32), and stained for flow cytometric analysis using combinations of the following antibodies: PeCy7-conjugated αCD4 and PerCP-Cy5.5-conjugated αTCR-β (T cells) and FITC-conjugated αCD45.2, αCD11b conjugated to PE, and Gr1 conjugated to PerCP-Cy5.5 (innate cells). Intracellular staining was performed as previously described (Ahern et al., 2010): cells were restimulated for 4 h with 0.1 μg/ml PMA (Sigma-Aldrich), 1 μg/ml ionomycin (Sigma-Aldrich), and 10 μg/ml Brefeldin A (Insight Biotechnology), washed, and stained for surface markers. Cells were then fixed overnight in eBioscience Fix/Perm buffer at 4°C. Cells were washed

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and permeabilized in permeabilization buffer (eBioscience) with 2% rat serum for 1 h at 4°C. Cells were then stained with αIL-17A conjugated to PE, αIFN-γ conjugated to APC or e450, and αKi-67 conjugated to FITC (BD) or appropriate isotype controls (BD) for 30 min at 4°C. ILCs were identified as previously described (Buonocore et al., 2010) using αThy1.2 PE-conjugated and FITC-conjugated αLy6A/E (Sca1) (both from BD Biosciences). Lineage$^+$ cells were excluded using PerCP-Cy5.5–conjugated αCD11b, αGr1, αB220, and αCD3e. For intracellular staining of ILCs, 10 μg/ml brefeldin A (Insight Biotechnology) was added for the last 4 h of overnight cultures in complete RPMI media. Cells were then processed similarly to T cells. For analysis of colonic T cells, CD4$^+$ TCRβ$^+$ cells were sorted from cLPL preparations according to the expression of the surface markers CD4 and TCRβ with a cell sorter (MoFlo; Dako) and restimulated overnight with 0.1 μg/ml PMA and 1 μg/ml ionomycin.

Cell culture. FACS sorted ILCs were cultured overnight in RPMI, 10% FCS, 100 U/ml penicillin/streptomycin, 2 mM L-glutamine, 0.05 mM β-mercaptoethanol (complete RPMI), and 10 ng/ml IL-1β. For Th17 differentiation from total CD4$^+$ T cells, splenic CD4$^+$ T cells were isolated from WT or *Il1r1*$^{-/-}$ mice and cultured in IMDM, 10% FCS, 100 U/ml penicillin/streptomycin, 2 mM L-glutamine, 0.05 mM β-mercaptoethanol (complete IMDM) in the presence of 2.5 ng/ml TGF-β, and 50 ng/ml IL-6 5, plus plate-bound αCD3 (2 μg/ml) and αCD28 (1 μg/ml). For Th17 differentiation from naive CD4$^+$ T cells, naive CD4$^+$ CD62L^{Hi} CD44^{low} CD25$^-$ were FACS sorted from the spleen and MLN of WT C57BL/6 or *Il23r*$^{-/-}$ mice and cultured for the indicated time in complete IMDM in the presence of 200 pg/ml TGF-β, 20 ng/ml IL-6, 20 ng/ml IL-1β, 10 ng/ml IL-21, 20 ng/ml IL-23, 10 μg/ml αIFN-γ and αIL-4, and plate-bound αCD3 and αCD28 (both at 5 μg/ml).

Quantification of gene expression by qRT-PCR. Quantitative real time PCR (qRT-PCR) was performed as previously described (Hue et al., 2006) with homogenization of frozen tissue samples performed using a FastPrep 24 homogenizer (MP Biomedicals) with lysing matrix D beads (MP Biomedicals). Primers for *Hprt* (Mm01545399_m1), *cxcl1* (Mm04207460_m1), *cxcl2* (Mm0436450_m1), *cxcl5* (Mm0436451_g1), *bcl2* (Mm0477631_m1), *bclxl* (Mm0437783_m1), *Il1r1* (Mm00434237_m1), and *rorc* (Mm01261022_m1) were obtained from Applied Biosystems. Levels of gene expression were normalized on *Hprt* expression, unless otherwise indicated. In-house primer sequences are as follows: CD3, forward, 5′-TTACAGAATGTGTGAAAACTGCATTG-3′, reverse, 5′-CACCAAGAGCAAGGAAGAAGATG-3′, and probe, 5′-ACATAGGCACCATATCCGGCTTTATCTTCG-3′; CCR3 forward, 5′-TGTTTACCTCAGTTCATCCACGG-3′, reverse, 5′-CAGAATGGTAATGTGAGCAGGAA-3′, and probe, 5′-TCTGCTCAACTTGGCCATCTCTGACC-3′; CCR5 forward, 5′-CATCGATTATGGTATGTCAGCACC-3′, reverse, 5′-CAGAATGGTAGTGTGAGCAGGA-3′, and probe 5′-TACCTGCTCAACCTGGCCATCTCTGA-3′; CCR6 forward, 5′-ACTCTTTGTCCTCACCCTACCG-3′, reverse, 5′-ATCCTGCAGCTCGTATTTCTTG-3′, and probe, 5′-ACGCTCCAGAACACTGACGCACAGTA-3′; CXCR6 forward, 5′-AAGCTGAGGACTCTGACAGATGTGT-3′, reverse, 5′-CCAAAAGGGCAGAGTACAGACAA-3′, and probe, 5′-CTGCTGAACTTGCCCCTGGCTGAC-3′; IL-23R forward, 5′-CCATCTGGATGATATAGTGATACCTTCT-3′, reverse, 5′-ATGGTCTTGGGTACAGTATCGTTTG-3′, and probe, 5′-CGTCCATCATTTCCAGGGCTCACACT-3′ (Ahern et al., 2010). Amplification was performed using TaqMan Fast Universal PCR Master Mix (Applied Biosystems).

Apoptosis assay. For in vitro assessment of apoptosis, total splenocytes from C57BL/6 or *Il1r1*$^{-/-}$ mice were depleted of CD8$^+$, MHC class II$^+$, Mac-1$^+$, and B220$^+$ cells by negative selection using sheep anti–rat–coated Dynabeads (Invitrogen; purity ≥82%). Enriched CD4$^+$ cells were cultured for 3 d with 2.5 μg/ml or 10 μg/ml each of plate-bound αCD3 and αCD28 (eBioscience). APC anti–Annexin-V antibody and 7-Aminoactinomycin (7AAD; eBioscience) were used for apoptosis assessment. All cells were acquired on a FACSCalibur (BD) or on a CyAn ADP flow cytometer (Beckman Coulter), and analysis performed using FlowJo (Tree Star) software.

Cytokine detection. For cytokine detection, colonic lamina propria cells we cultured overnight in complete RPMI media. IL-6, IFN-γ, IL-17, TNF, and IL-1β levels in culture supernatants were measured using FlowCytomix Bead-based assay (eBioscience) and cytokine concentration was normalized to cell number. To assess innate lymphoid cell function, colonic lamina propria cells were cultured with or without recombinant IL-23 (10 ng/ml; R&D) overnight, and IL-17 and IFN-γ were measured in the supernatants as above. Organ explants were prepared as previously described (Hue et al., 2006) and cultured overnight in complete RPMI media. IL-1β levels in the supernatants were determined by Bio-Plex Cytokine assay (Bio-Rad Laboratories), and concentrations were normalized to the weight of the explants.

Statistics. The nonparametric Mann-Whitney test was used for assessment of statistical significance of in vivo experiments. The Student's *t* test was used to assess the significance of in vitro experiments. Weight curves were compared using two-way ANOVA. Data were considered significant when $P < 0.05$.

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